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Phylogenetic relationships in the family *Streptomycetaceae* using multi-locus sequence analysis

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Abstract

The family *Streptomycetaceae*, notably species in the genus *Streptomyces*, have long been the subject of investigation due to their well-known ability to produce secondary metabolites. The emergence of drug resistant pathogens and the relative ease of producing genome sequences has renewed the importance of *Streptomyces* as producers of new natural products and resulted in revived efforts in isolating and describing strains from novel environments. A previous large study of the phylogeny in the *Streptomycetaceae* based on 16S rRNA gene sequences provided a useful framework for the relationships among species, but did not always have sufficient resolution to provide definitive identification. Multi-locus sequence analysis of 5 house-keeping genes has been shown to provide improved taxonomic resolution of *Streptomyces* species in a number of previous reports so a comprehensive study was undertaken to evaluate evolutionary relationships among

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species within the family *Streptomycetaceae* where type strains are available in the ARS Culture Collection or genome sequences are available in GenBank. The results of the analysis supported the distinctiveness of *Kitasatospora* and *Streptacidiphilus* as validly named genera since they cluster outside of the phylogenetic radiation of the genus *Streptomyces*. There is also support for the transfer of a number of *Streptomyces* species to the genus *Kitasatospora* as well for reducing at least 31 species clusters to a single taxon. The multi-locus sequence database resulting from the study is a useful tool for identification of new isolates and the phylogenetic analysis presented also provides a road map for planning future genome sequencing efforts in the *Streptomycetaceae*.

Keywords

MLSA; *Streptomyces* ; *Kitasatospora* ; *Streptacidiphilus* ; Systematics

Introduction

Species within the family *Streptomycetaceae*, notably those in the genus *Streptomyces,* have been the subject of investigations for over a century, particularly following the discovery of their prodigious ability to produce antibiotics. The importance and role of *Streptomyces* in nature and biotechnology have been thoroughly reviewed elsewhere and thus will not be discussed here. However it was noted that 58 new *Streptomyces* species have been described to date since 2012 and *Streptomyces* were mentioned more than 22,000 times in the patent literature worldwide during the same period. The emergence of multiple drug resistant pathogens and relative ease of generating draft whole genome sequences from microorganisms has elevated the importance of *Streptomyces* strains as producers of valuable new natural products and resulted in renewed efforts in isolating and describing strains from new and novel environments. As thoroughly discussed by Labeda et al. (2012) in their study of the 16S rRNA gene phylogeny of the *Streptomycetaceae*, the systematics and taxonomy of the members of the family have had a long and meandering history, from one based largely on morphological characteristics, through numerical taxonomic analysis of large numbers of physiological (i.e., phenotypic) traits, and finally on to use of molecular genetic methods such as DNA:DNA hybridization and 16S rRNA gene sequencing. The 16S rRNA gene phylogeny provides a useful framework for inferring the relationships between species, but did not always seem to have enough taxonomic resolution to provide definitive identifications in the absence of DNA–DNA relatedness data. The observation that species of the genera *Kitasatospora* and *Streptacidiphilus* were found within the phylogenetic radiation of *Streptomyces* based on 16S rRNA gene sequences led some to question the validity of these genera (Kämpfer 2012). The use of multi-locus sequence analysis (MLSA) has been clearly demonstrated to provide the necessary resolution, comparable to that of DDH, for species discrimination within the *Streptomycetaceae* (Guo et al. 2008; Rong et al. 2009, 2010; Rong and Huang 2010, 2012, 2014). MLSA has been used recently to clarify the taxonomic position of members of a number of the 16S rRNA gene phylogeny clades including Clade 126, the *Streptomyces albus* clade (Labeda et al. 2014), and Clade 6, the *Streptomyces hirsutus* clade (Labeda et al. 2016), as well as a study of the strains within the NRRL Culture Collection identified as *Streptomyces scabiei* (Labeda 2016). A comprehensive study was therefore undertaken to evaluate the phylogenetic relationship of

all taxa within the family *Streptomycetaceae* whose type strains were currently available in the ARS (NRRL) Culture Collection, as well as those type strains having draft genome sequences available in the public databanks.

Methods

The strains sequenced in the study were obtained from the ARS (NRRL) Culture Collection, Peoria, IL, USA where they had been maintained in long-term storage as lyophilized stocks and are listed in Supplemental Table S1. Strains were cultivated on yeast extract-malt extract agar (YM) ISP-2 medium (Shirling and Gottlieb 1966) at 28 °C for generation of biomass for DNA extraction. Some data were obtained from previous MLSA studies of from genome sequences deposited in NCBI for strains sequenced elsewhere.

Genomic DNA was isolated from all strains using UltraClean®Microbial DNA isolation kits (MoBio Labs, Carlsbad, CA) following the instructions of the manufacturer. Partial sequences of the house-keeping genes *atpD* (ATP synthase F1, beta subunit), *gyrB* (DNA gyrase B subunit), *rpoB* (RNA polymerase beta subunit), *recA* (recombinase A) and *trpB* (tryptophan synthetase, beta subunit) were amplified and sequenced using the primers and protocols described previously by Labeda et al. (2014). Amplified products were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA), sequenced using BigDye 3.1 on an ABI model 3730 sequencer and assembled using Sequencher version 5.2 (Gene Codes, Ann Arbor, MI). The gene sequences for the 5 house-keeping loci for strains sequenced locally were deposited in Genbank (see Supplemental Table S1).

The draft genome sequences of a number of strains were also determined in the course of the present study. Libraries were prepared with genomic DNA isolated as described above using a Nextera XT DNA library preparation kit (Illumina, San Diego, CA) following the manufacturer's instructions. The library preparations were sequenced with a MiSeq Desktop Sequencer (Illumina, San Diego, CA) and the resulting short sequence reads were trimmed for quality and removal of adapter sequences, and subsequently *de novo* assembled with CLC bio Genomics Workbench version 8.0.1 (CLC bio, Waltham, MA). The draft genome sequences have been deposited in the NCBI Whole Genome Shotgun database (See Supplementary Table S2).

The gene sequences for the 5 house-keeping loci for strains sequenced locally were organised using Bacterial Isolate Genomic Sequence Database (BIGSdb) version 1.12.3 (Jolley and Maiden 2010) on the ARS Microbial Genomic Sequence Database server (https:// 199.133.98.43). Where available (see Supplemental Table S2), genome sequences were up-loaded into the sequence bin for the respective strains in the BIGSdb isolate database. The genome sequences were scanned within BIGSdb for house-keeping loci, the specific sequence regions were tagged and the allele sequences and respective allele designations added to the sequence database if new alleles were found. The strain record was then updated with the matching allele id for each locus in the strain database. The sequences for the alleles of the loci for all strains in the study were individually aligned with MAFFT (Katoh and Standley 2013), subsequently concatenated head to tail in-frame, and exported in FASTA format, providing a dataset of 692 strains and 2608 positions.

Phylogenetic relationships were constructed from the partitioned gene data in IQ-Tree version 1.41 (Nguyen et al. 2015) using the Maximum Likelihood based on the General Time Reversible model (Nei and Kumar 2000), GTR + F + I + G4, which had been determined to be the optimal model for these data during the model test phase of analysis. The trees were subjected to 1000 ultrafast bootstrap replications (Minh et al. 2013) followed by 1000 replications of assessment of branch supports with single branch tests with SH-like approximate likelihood ratio test (Guindon et al. 2010).

MLSA evolutionary distances were determined using MEGA 6.0 (Tamura et al. 2013) by calculating the Kimura 2-parameter distance (Kimura 1980) and are shown in Supplemental Table S3 for each strain pair. Strain pairs having ≤0.007 MLSA evolutionary distance were considered conspecific based on the guideline empirically determined by Rong and Huang (2012) that this MLSA distance (Kimura 2-paramenter distance) computed from the partial sequences of these house-keeping loci equates to 70% DNA–DNA homology.

Results and discussion

The phylogenetic relationship of species within the *Streptomycetaceae* based on the partial sequences of the 5 house-keeping loci can be seen in Fig. 1a–j. The node labels also include the taxon's clade designation from the 16S rRNA gene sequence phylogenetic study of Labeda et al. (2012) as well as the clade assignment in the numerical taxonomic study of Williams et al. (1983), if included in that study. The clades designated on the trees were defined based on an MLSA distance ≤0.007 (shown in Supplemental Table S3), indicating that these represented the same species. Bootstrap values less than 95% or branches having SH-aLRT less than 85% were not shown on the tree as recommended by the developers of IQ-Tree.

There is generally good correlation between the phylogenetic relationships observed between species in the individual clades (Fig. 1a–j) with those observed in our previous phylogenetic study based on 16S rRNA gene sequences (Labeda et al. 2012). Not surprisingly, there is also fairly good correlation within clades to those assigned in the numerical taxonomic study of Williams et al. (1983), which could have been expected since the MLSA study should correlate well with observed phenotype. A major difference from the phylogenetic tree constructed using 16S rRNA sequence data is that those strains identified as species within the genera *Kitasatospora* and *Streptacidiphilus* are clearly observed (Fig. 1a) to fall outside of the radiation of the genus *Streptomyces*, thus confirming their validity and making the proposal of Kämpfer (2012) that these are genera *incertae sedis* unlikely. Girard and colleagues previously reported (Girard et al. 2014) evolutionary genetic changes in conserved developmental genes between *Kitasatospora* and *Streptomyces* that supported the validity of the genus *Kitasatospora* and their proposal was confirmed not only by the phylogenetic relationships shown in Fig. 1a but by a scan of the genomes held in the ARS Microbial Genomic Sequence database (http://199.133.98.43) in the present study that demonstrated that the *bldB* gene locus was absent in strains identified as *Kitasatospora* or *Streptacidiphilus* as they suggested. The 5-gene phylogeny shown in Fig. 1a also makes it quite evident that the type strains of many validly named *Streptomyces* species clearly belong within the genus *Kitasatospora* including: *Streptomyces aburaviensis* Nishimura

et al. 1957, *Streptomyces albolongus* Tsukiura et al. 1964, *Streptomyces aureofaciens* Dugger 1948 (with *Streptomyces avellaneus* Baldacci and Grein 1966 as a later synonym), *Streptomyces chrysomallus* subsp. *fumigatus* Frommer 1959, *Streptomyces cinereorectus* Terekhova and Preobrazhenskaya 1986, *Streptomyces herbaricolor* Kawato and Shinobu 1959, *Streptomyces misakiensis* Nakamura 1961, *Streptomyces psammoticus* Virgilio and Hengeller 1960, and *Streptomyces purpeofuscus* Yamaguchi and Saburi 1955. Strain NRRL B-1588^T, the type strain of *Streptomyces cinnamonensis* subsp. *cinnamonensis*, was also unexpectedly found within the *Kitasatospora* clade but equivalent type material from another culture collection must be obtained and analysed before any proposal is made to reclassify this strain. It is interesting to note that NRRL B-16,504, a strain deposited into the ARS Culture Collection as a proposed new species '*Kitasatospora papulosa*' but demonstrated as a member of the species *Streptomyces pratensis* in this study (Fig. 1i) was found to contain an allele of the *bldB* locus. *Streptacidiphilus* species form a distinct, well supported clade that contains all of the taxa for which sequences were available. Strain NRRL B-24555, an isolate from cave soil collected on Jeju Island, Korea and deposited by S. D. Lee as the type strain of a possible new species to be named *Streptacidiphilus jeojiense* but never published, appears distinct from the other *Streptacidiphilus* species sequenced and likely represents a new species in this genus but further characterisation of morphological, chemotaxonomic and physiological traits will be necessary to fully describe it.

It is notable that both strains of *Streptomyces griseoplanus* that were included in the present study, representing the type strain and the International *Streptomyces* Project (ISP) strain, appear to be phylogenetically distinct from the genera *Kitasatospora*, *Streptacidiphilus* while also lacking the gene *bldB* and outside of the phylogenetic radiation of *Streptomyces* sensu stricto. Although a draft genome sequence has been determined for the type strain (NRRL B-3064^T), further morphological, chemotaxonomic, and physiological study is necessary to formally propose a new genus for these strains. Likewise it will be interesting for future studies to address the relationships of the type species of the recently described genus *Allostreptomyces* (Huang et al. 2016) to other genera of the family *Streptomycetaceae* using whole genome sequencing and MLSA.

It is interesting to observe that certain morphological traits of *Streptomyces* species correlate with phylogenetic relationships observed from MLSA, including the clustering of those taxa having rugose spore surface ornamentation into a single well supported clade (Fig. 1a), while those exhibiting verticillate sporophore structures, originally described as species within the genus *Streptoverticillium*, are also found within another well-supported clade (Fig. 1c). Both clades are found within the radiation of the genus *Streptomyces*, as currently defined, and any proposal toward subdividing *Streptomyces* will require the availability of far more genome sequences for representative type strains for analysis.

Many of the clades presented in Fig. 1a–j illustrate species synonymies for which proposals have already been published. These include: *Streptomyces albidoflavus* (Rong et al. 2009) with *Streptomyces canescens*, *Streptomyces champavatii*, *Streptomyces coelicolor*, *Streptomyces felleus*, *Streptomyces globisporus* subsp. *caucasicus*, *Streptomyces griseus* subsp. *solvifaciens*, *Streptomyces limosus*, *Streptomyces odorifer* and *Streptomyces sampsonii* as later heterotypic synonyms, with the addition of *Streptomyces galilaeus*

CGMCC 4.1320^T, *Streptomyces sioyaensis* CGMCC 4.1306^T and *Streptomyces vinaceus* CGMCC 4.1305^T in the present study (Fig. 1a); *Streptomyces albovinaceus* (Rong and Huang 2010), with *Streptomyces griseinus* and *Streptomyces mediolani* as later heterotypic synonyms, now includes *S. globisporus* subsp. *globisporus* NRRL B-2872^T in the present study (Fig. 1i) which has priority over *S. albovinaceus* requiring an emended description after genome sequences are obtained for all and DDH determinations are performed; *S. albus* (Labeda et al. 2012) with *Streptomyces almquistii*, *Streptomyces flocculus, Streptomyces gibsonii* and *Streptomyces rangoonensis* as later heterotypic synonyms (Fig. 1b); *Streptomyces anulatus* (Rong and Huang 2010) with *Streptomyces praecox* as a later heterotypic synonym including *Streptomyces chrysomallus* subsp. *chrysomallus* NRRL 2250^T and ATCC 11523^T in the present study (Fig. 1j), confirming the observation of Lanoot et al. (2005); *Streptomyces atroolivaceus* (Rong and Huang 2010) with *Streptomyces olivaceoviridis* as a later heterotypic synonym (Fig. 1i); S*treptomyces bacillaris* (Rong and Huang 2010) with *Streptomyces griseobrunneus* as a later heterotypic synonym, as well as *Streptomyces cavourensis* subsp. *cavourensis* NRRL ISP-5300^T in the present study (Fig. 1i); *Streptomyces castelarensis* (Rong and Huang 2012) with *Streptomyces antimycoticus* and *Streptomyces sporoclivatus* as later heterotypic synonyms including *Streptomyces mordarskii* NRRL B-1346^T in the present study (Fig. 1a); *Streptomyces cyaneofuscatus* (Rong and Huang 2010) with *Streptomyces cavourensis* subsp. *washingtonensis* as a later heterotypic synonym, but not *Streptomyces fluorescens* as proposed by Rong and Huang (2010) (Fig. 1j); *Streptomyces ehimensis* (Rong and Huang 2012) with *Streptomyces luteoverticillatus* as a later heterotypic synonym (Fig. 1c); *Streptomyces fimicarius* (Rong and Huang 2010) with *Streptomyces acrimycini*, *Streptomyces baarnensis*, *Streptomyces caviscabies* and *Streptomyces flavofuscus* as later heterotypic synonyms, including *Streptomyces bohaiensis* NRRL B-24956^T in the present study (Fig. 1j); *Streptomyces flavovirens* (Rong and Huang 2010) with *Streptomyces flavogriseus* as a later heterotypic synonym including *Streptomyces nigrifaciens* NRRL B-2094^T in the present study (Fig. 1i); *S. griseus* (Rong and Huang 2010) with *Streptomyces erumpens*, *'Streptomyces ornatus'* and *Streptomyces setonii* as later heterotypic synonyms (Fig. 1j); *Streptomyces hirsutus* (Labeda et al. 2016) with *Streptomyces cyanoalbus* as a later heterotypic synonym (Fig. 1f); *Streptomyces hygroscopicus* (Rong and Huang 2012) with *Streptomyces demainii*, *Streptomyces endus* and *Streptomyces sporocinereus* as later heterotypic synonyms (Fig. 1a); *Streptomyces javensis* (Rong and Huang 2012) with *Streptomyces yogyakartensis* as a later heterotypic synonym (Fig. 1a); *Streptomyces microflavus* (Rong and Huang 2010) with *Streptomyces alboviridis*, *S. griseus* subsp. *alpha*, *S. griseus* subsp. *cretosus* and *Streptomyces luridiscabiei* as later heterotypic synonyms including *Streptomyces lipmanii* NRRL B-1229^T, *Streptomyces willmorei* NRRL B-1332^T (confirming the proposals of Lanoot et al. (2005) and Labeda et al. (2014)) and *Streptomyces thioluteus* NRRL B-1667^T in the present study (Fig. 1j); *Streptomyces nigrescens* (Rong and Huang 2012) with *Streptomyces libani* subsp. *libani* as a later heterotypic synonym; *Streptomyces prasinopilosus* (Labeda et al. 2016) with *Streptomyces emeiensis* as a later heterotypic synonym (Fig. 1f); *Streptomyces prasinus* (Labeda et al. 2016) with *Streptomyces bambergiensis* as a later heterotypic synonym (Fig. 1f); *Streptomyces puniceus* (Rong and Huang 2010) with *Streptomyces californicus* and *Streptomyces floridae* as later heterotypic synonyms (Fig. 1i); *Streptomyces rectiverticillatus* (Rong and Huang 2012)

with *Streptomyces aureoversilis* as a later heterotypic synonym (Fig. 1c); *Streptomyces rhizosphaericus* (Rong and Huang 2012) with *Streptomyces asiaticus*, *Streptomyces cangkringensis* and *Streptomyces indonesiensis* as later heterotypic synonyms (Fig. 1a).

This phylogenetic study also supported proposals in previous studies for elevating former subspecies to new species, including *Streptomyces glebosus*, formerly *Streptomyces hygroscopicus* subsp. *glebosus* (Rong and Huang 2012) (Fig. 1b), *Streptomyces ossamyceticus*, formerly *S. hygroscopicus* subsp. *ossamyceticus* (Rong and Huang 2012) (Fig. 1h) and *Streptomyces pathocidini*, formerly *S. albus* subsp. *pathocidicus* (Labeda et al. 2014) (Fig. 1b).

In the present study some potentially misidentified collection strains were identified based on phylogenetic position and an MLSA distance ≤0.007. *Streptomyces narbonensis* NRRL ISP-5016^T is identified as a strain of *S. albus* (see Fig. 1b) while the original type strain, NRRL B-1680^T is found nearest to *Streptomyces zaomyceticus* NRRL B-2038^T (near *S. venezuelae* in Fig. 1c). As mentioned by Labeda et al. (2012), '*S. albus*' J1074, whose genome has been sequenced, is actually a strain of *Streptomyces albidoflavus* and is found in that clade in Fig. 1i. It is also quite evident that *Streptomyces lydicus* NRRL ISP-5461 is actually a strain of *Streptomyces varsoviensis* because all share identical alleles for the 5 house-keeping loci (Fig. 1c) and are phylogenetically very distant from *Streptomyces lydicus* CGMCC 4.1412^T (Fig. 1b).

It should also be noted that *Streptomyces fulvissimus* DSM 40593^T (added from a draft genome sequence) is found in the *Streptomyces microflavus* clade (Fig. 1j), quite distant from *Streptomyces fulvissimus* NRRL B-1453^T (near *Streptomyces alboflavus* in Fig. 1d) that was sequenced locally. It is not clear in this case which strain represents the actual type of *Streptomyces fulvissimus* and comparison with the type strain acquired from one or more other culture collections will be necessary to clarify this situation.

The relationships shown in the maximum likelihood phylogenetic tree in Fig. 1a–j, constructed from the alignment resulting from concatenation of the partial sequences of the 5 house-keeping loci, where the observed MLSA distances between clade members is also ≤0.007 suggest that a number of validly named *Streptomyces* species should be considered synonymous although further studies, including the preparation of draft genome sequences and determination of genomic relatedness, are needed to confirm any merging of these taxa.

To simplify the discussion of the results illustrated in this very long phylogenetic tree (Fig. 1), observations on each page of the figure will be discussed in order.

In Fig. 1a, aside from the previously discussed species to be reassigned to *Kitasatospora*, it was observed that *Streptomyces samsunensis* NRRL B-24803^T represents a later heterotypic synonym of *Streptomyces malaysiensis* NRRL B-24313^T while *Streptomyces cuspidosporus* NRRL B-5620^T represents a later heterotypic synonym of *Streptomyces sparsogenes* NRRL 2940^T. The equivalent strains of *'Streptomyces cattleya'* (NRRL 8057 and DSM 46488) that have genome sequences available in the public databanks clearly represent a distinct species but further morphological, chemotaxonomic and phenotypic characterisation is necessary to prepare a formal species description.

In Fig. 1b, the phylogenetic analysis and observed MLSA distance (0.002) confirmed that *Streptomyces aminophilus* NRRL ISP-5186^T is a later heterotypic synonym of *Streptomyces cacaoi* subsp. *cacaoi* NRRL B-1220^T as proposed earlier by Lanoot et al. (2002) based on SDS-PAGE of proteins. It can also be seen that *Streptomyces ochraceiscleroticus* CGMCC 4.1096^T is equivalent to both *S. ochraceiscleroticus* NRRL ISP-559^T and *Streptomyces violens* NRRL ISP-5597^T but not *S. violens* CGMCC 4.1786^T which will require sequencing of additional type strains of *S. violens* from other collections to clarify the taxonomic status of this species.

In Fig. 1c, it was confirmed that *Streptomyces spitsbergensis* NRRL B-24285^T is a later synonym of *Streptomyces baldaccii* NRRL B-3500^T as proposed by Hatano et al. (1997) but the present study supports the revival of *Streptomyces griseoverticillatus* because it does not appear to represent a later synonym of *Streptomyces cinnamoneus* as Hatano et al. (1997) proposed. *Streptomyces sapporonensis* DSM 41675^T appears to be a later synonym of *S. griseoverticillatus* DSM 40507^T. It can be observed that *Streptomyces alboverticillatus* NRRL B-24281^T has the later synonyms *Streptomyces griseocarneus* NRRL B-1350^T and *Streptomyces septatus* NRRL ISP-5577^T, *Streptomyces abikoensis* CGMCC 4.1662^T has the later synonym *Streptomyces kashmirensis* NRRL B-3103^T and *Streptomyces mashuensis* DSM 40221^T has the later synonym *Streptomyces kishiwadensis* NRRL B-12326^T. *Streptomyces cinnamoneus* subsp. *albosporus* NRRL B-5624 appears to represent a distinct species and *S. cinnamoneus* subsp. *lanosus* NRRL B-24290 and *S. cinnamoneus* subsp. *sparsus* NRRL B-24291 appear to represent a single new species but it is necessary to determine draft genome sequences for all of the *Streptomyces cinnamoneus* subspecies for genomic DDH comparisons along with morphological and physiological characterisation before these formal descriptions can be prepared.

In Fig. 1d, it was confirmed that *Streptomyces laceyi* CGMCC 4.1832^T and *Streptomyces spheroides* NRRL 2449^T represent later synonyms of *Streptomyces niveus* NRRL 2466^T as proposed by Tamura et al. (2008). It was also confirmed that *Streptomyces fradiae* NRRL B-1195^T is an earlier synonym of *Streptomyces roseoflavus* NRRL B-2789^T as proposed by Lanoot et al. (2004). This section of the tree supports that *Streptomyces aureocirculatus* NRRL B-3324^T (and NRRL ISP-5386^T) has the later synonym *Streptomyces glomeroaurantiacus* NRRL B-3375^T, *Streptomyces coerulescens* CGMCC 4.1597^T has the later synonym *Streptomyces coeruleofuscus* NRRL B-5417^T and *Streptomyces angustmyceticus* CGMCC 4.0207^T has the later synonym *Streptomyces pluricolorescens* CGMCC 4.0236^T. The status of strain *Streptomyces noursei* CGMCC 4.0213^T is unclear because two other type strains of *S. noursei* are found in the tree at the top of Fig. 1c.

In Fig. 1e it was confirmed that *Streptomyces flaviscleroticus* NRRL B-12173^T is a later synonym of *Streptomyces minutiscleroticus* NRRL B-12202^T as proposed by Lanoot et al. (2005). The following observations can also be made: *Streptomyces pseudo-griseolus* NRRL B-3288^T has the later synonyms *Streptomyces gancidicus* NRRL B-1872^T, *Streptomyces nashvillensis* NRRL B-2606^{T}and *Streptomyces rubiginosus* NRRL B-3983^T; *Streptomyces althioticus* NRRL B-3981^T has the later synonyms *Streptomyces griseorubens* NRRL B-3982^T, *Streptomyces matensis* NRRL B-2576^T and *Streptomyces*

phaeogriseichromatogenes NRRL 2834^T; *Streptomyces arabicus* NRRL B-1733^T has the later synonyms *Streptomyces erythrogriseus* NRRL B-3808^T, *Streptomyces griseoincarnatus* NRRL B-5313^T and *Streptomyces variabilis* NRRL B-3984^T; *Streptomyces pilosus* NRRL ISP-5097^T has the later synonym *Streptomyces flavoviridis* NRRL ISP-5153^T; *Streptomyces asterosporus* NRRL B-24328^T has the later synonym *Streptomyces aureorectus* NRRL B-24301^T; *Streptomyces coelescens* NRRL B-12348^T has the later synonyms *Streptomyces anthocyanicus* NRRL B-24292^T, *Streptomyces humiferus* NRRL B-3088^T, *Streptomyces rameus* NRRL B-16924^T, *Streptomyces sannanensis* NRRL B-24303^T and *Streptomyces violaceorectus* NRRL B-12181^T. It is noted that *'Streptomyces coelicolor'* JI 1147 and *'Streptomyces lividans'* TK24 are also strains of *S. coelescens*. In addition, *Streptomyces ennisocaesilis* NRRL B-16365^T, *Streptomyces geysiriensis* NRRL B-12102^T and *Streptomyces vinaceusdrappus* NRRL ISP-5169^T represent later synonyms of *Streptomyces rochei* NRRL B-2410^T while *Streptomyces daghestanicus* NRRL B-5418^T is a later synonym of *Streptomyces griseoviridis* NRRL ISP-5229^T.

In Fig. 1f it should be noted that the thermophilic *Streptomyces* species group into a single clade, continued onto Fig. 1g. It was observed that *Streptomyces roseodiastaticius* CGMCC 4.1788^T has the later synonyms *Streptomyces tricolor* NRRL B-16925^T (as proposed by Lanoot et al. 2004) and *Streptomyces bangladeshensis* NRRL B-24326^T. The following observations can also be made: *Streptomyces olivaceoviridis* NRRL B-12280^T has the later synonym *Streptomyces corchorusii* NRRL B-12289^T (also DSM 40340^T); *Streptomyces murinus* NRRL B-2286^T has the later synonyms *Streptomyces costaricanus* NRRL B-16897^T and *Streptomyces griseofuscus* NRRL B-5429^T; *Streptomyces viridosporus* NRRL ISP-5243^T has the later synonym *Streptomyces ghanaensis* NRRL B-12104^T (also ATCC 14672^T); *Streptomyces griseomycini* NRRL B-5421^T has the later synonyms *Streptomyces graminearus* NRRL B-16369^T and *Streptomyces griseostramineus* NRRL B-5422^T; *Streptomyces thermovulgaris* NRRL B-12375^T has the later synonym *Streptomyces thermonitrificans* NRRL B-12534^T.

In Fig. 1g it can be observed that *Streptomyces phaeopurpureus* NRRL B-2260^T (and DSM 40125^T) is confirmed as a later synonym of *Streptomyces griseorubiginosus* CGMCC 4.1766^T (and DSM 40469^T) as proposed in Gauze et al. (1983). The following observations can also be made: *Streptomyces inusitatus* NRRL B-16929^T has the later synonym *Streptomyces longwoodensis* NRRL B-16923^T (=DSM 41677^T); *Streptomyces clavifer* CGMCC 4.1064^{T}has the later synonyms *Streptomyces canus* NRRL B-3980^T (=DSM 40017^T) and *Streptomyces ciscaucasicus* NRRL ISP 5275^T (=DSM 40275^T).

In Fig. 1h it was observed that *Streptomyces goshikiensis* NRRL B-5428^T has the later synonym *Streptomyces sporoverrucosus* NRRL B-16379^T and *Streptomyces melanogenes* NRRL B-2072^T has the later synonym *Streptomyces noboritoensis* NRRL B-12152^T.

Most relationships in Fig. 1i and 1j have already been discussed earlier with the exception of the observation that *Streptomyces griseolus* (both CGMCC 4.1864^T and NRRL B-2925^T) is a later synonym of *Streptomyces halstedii* NRRL B-1238^T and NRRL ISP-5016^T) based on presence of identical alleles for all 5 house-keeping loci. It should be noted that

Streptomyces graminofaciens CGMCC 4.1359^T (bottom of Fig. 1h) is shown not to be a later synonym of *Streptomyces halstedii* as was suggested by Rong and Huang (2010).

The phylogenetic analyses performed during the course of this study (see Fig. 1a) support the transfer of those *Streptomyces* species found within the radiation of the genus *Kitasatospora* to that genus and emended descriptions for these follow below. Although it would be optimal to also have phenotypic properties to support the proposed taxonomic changes, no useful traits have been discovered in past studies and gene sequencing capabilities have become ubiquitous and within the reach of most investigators so a gene phylogeny-based classification should be completely acceptable. The taxonomic status of *Streptomyces chrysomallus* subsp. *fumigatus* has been problematic since the Lanoot et al. (2005) proposal that *S. chrysomallus* subsp. *chrysomallus* is a later is synonym of *S. anulatus* which is also supported by the present study (see Fig. 1j). *S. chrysomallus* subsp. *fumigatus* is phylogenetically distant from *S. chrysomallus* subsp. *chrysomallus* and represents a new species within *Kitasatospora* for which the name *Kitasatospora fumigata* is proposed below.

The results presented in this study demonstrate the value of MLSA using partial sequences of single-copy house-keeping genes to provide resolution of taxonomic issues in the *Streptomycetaceae* and could result in a 116 species reduction from the over 780 *Streptomyces* species currently listed on the List of Prokaryote Names With Standing in Nomenclature (www.bacterio.net). This method has been applied to over 400 uncharacterised strains in the NRRL Culture Collection to date and at least 20 potentially new species have been discovered among the microbial strains collected since the 1950s (Labeda, *unpublished obervations*). Although there could be criticism of the taxonomic resolution and accuracy in utilising only the limited set of 5 house-keeping genes in the present study, our experience has shown that these loci provide an excellent assessment of the phylogenetic relationships between species in the *Streptomycetaceae*. Moreover, the phylogenetic relationships of 170 strains based on maximum likelihood analysis of the sequences of the 5 genes utilised in this study shows good correlation (See Supplemental Figure S1a–d) with that determined utilizing 1487 core genes (1943,267 bp) extracted from the genome sequences of these strains using the Genome Comparator function of BIGSdb, utilising the well-annotated genome sequence (Genbank FN554889) for *S. scabiei* RL87.22 (=NRRL B-24449) as the reference and with the core gene threshold set at 90%. Although the number of draft or finished genome sequences for strains in the *Streptomycetaceae* is continually growing, totalling more than 770 at this time, only about 182 of these are from type strains making definitive whole genome or core gene molecular systematics of the entire family *Streptomycetaceae* not yet within reach. The alleles of the house-keeping genes utilised in this study can be easily discovered within draft or finished genomes and added to the growing sequence database using the genome scanning capability within the BIGSdb software, making it possible to positively and correctly identify incorrectly named genome strains, such as classifying J1074 as a strain of *S. albidoflavus* rather than *S. albus* as reported in the genome databases. Furthermore this database can expanded to include all of the relevant single-copy core genes for the family *Streptomycetaceae* once there are genome sequences available for a representative set of type strains. A major value of the phylogenetic relationships illustrated in Fig. 1a–j, aside from providing taxonomic insight

into the genus *Streptomyces*, is in providing a guide map for selection and prioritising critical species within the *Streptomycetaceae* for future genome sequencing efforts, as well as highlighting those strains requiring genome sequencing because taxonomic proposals of synonyminity need DDH between strains for confirmation, including many of those identified above. The multigene database has been demonstrated as an important tool for identifying phytopathogenic *Streptomyces* species when 16 of 43 strains of putative *S. scabiei* strains in the ARS Culture Collection were confirmed to represent non-pathogenic species while 6 strains possibly represent new phytopathogens (Labeda 2016). We have already demonstrated the usefulness of utilising the genomic sequence database, and the associated phylogeny constructed from the data stored in it, to discover new antibiotic producing strains as well as novel secondary metabolites (Price et al. 2016) in the course of discovery of the new tunicamycin analog, quinvosomycin, and its producing strain. The addition of substantially more *Streptomyces* genomes and expansion of the gene set used for phylogenetic analysis, aside from providing for a phylogenomic revision of the systematics of the *Streptomycetaceae*, should make this database an invaluable tool for mining these genomes for hitherto undiscovered natural products.

The ARS Microbial Genomic Sequence Database database is available at http://199.133.98.43/. The new names proposed as a result of this study are described below.

Description of *Kitasatospora aburaviensis* comb. nov.

Kitasatospora aburaviensis (a.bu.ra.vi.en'sis. N.L. fem. adj. *aburaviensis* of or belonging to Aburabi, Shiga Prefecture, Japan, the source of the soil from which the microorganism was isolated).

Basonym *Streptomyces aburaviensis* Nishimura, Kimura, Tawara, Sasaki, Nakajima, Shimaoka, Okamoto, Shimohira and Isono 1957^{AL}.

The description is that reported in Kämpfer (2012).

The type strain is AS 4.1469^{T}, ATCC 23869^{T}, BCRC 11617^{T}, CBS 280.60^{T}, CBS 608.68^{T}, CCRC 11617^{T}, CECT 3315^{T}, CGMCC 4.1469^{T}, DSM 40033^{T}, IFO 12830^{T}, IMET 43031^{T}, IMET 43081^{T}, ISP 5033^{T}, JCM 4170^{T}, JCM 4613^{T}, KACC 20033^{T}, KCTC 9663^{T}, LMG 19305^{T}, NBRC 12830^{T}, Nishimura S-66^{T}, NRRL B-2218^{T}, NRRL-ISP 5033^{T}, VKM Ac-1868^{T}.

Description of *Kitasatospora albolonga* comb. nov.

Kitasatospora albolonga (al.bo.lon'ga. L. adj. *albus* white; L. adj. *longus* long; N.L. fem. adj. *albolonga* white and long).

Basonym *Streptomyces albolongus* Tsukiura, Okanishi, Koshiyama, Ohmori, Miyaki and Kawaguchi 1964^{AL}.

The description is that reported in Shirling and Gottlieb (1972).

The type strain is AS 4.1661^{T}, ATCC 27414^{T}, Bristol-Banyu 304R7^{T}, CBS 766.72^{T}, CGMCC 4.1661^{T}, DSM 40570^{T}, IFO 13465^{T}, ISP 5570^{T}, JCM 4716^{T}, KCTC 9676^{T}, KCTC 9749^{T}, NBRC 13465^{T}, NRRL B-3604^{T}, NRRL ISP-5570^{T}, RIA 1426^{T}, VKM Ac-704^{T}.

Description of *Kitasatospora aureofaciens* comb. nov.

Kitasatospora aureofaciens (au.re.o.fa'ci.ens. L. adj. *aureus* golden; L. part. adj. *faciens* producing; L. part. adj. *aureofaciens* producing golden (referring to pigment produced by the vegetative mycelium of the microorganism).

Basonym *Streptomyces aureofaciens* Duggar 1948, 177^{AL}; later synonym *Streptomyces avellaneus* Baldacci and Grien 1966, 195^{AL}.

The descriptions is that reported in Kämpfer (2012).

The type strain is ATCC 10762^{T}, ATCC 23884^{T}, CBS 434.51^{T}, CECT 3206^{T}, CGMCC 4.0568^{T}, CIP 57.11^{T}, DSM 40127^{T}, IFO 12594^{T}, IFO 12843^{T}, IMET 43577^{T}, ISP 5127^{T}, JCM 4008^{T}, JCM 4624^{T}, KACC 20180^{T}, Lederle Labs A-377^{T}, LMG 5968^{T}, NBRC 12594^{T}, NCIB 8234^{T}, NRRL ISP-5127^{T}, RIA 1129^{T}, Waksman 3708^{T}.

Description of *Kitasatospora cinereorecta* comb. nov.

Kitasatospora cinereorecta (ci.ne.re.o.rec'ta. L. adj. *cinereus* similar to ashes, ash-colored; L. adj. *rectus* straight; N.L. fem adj. *cinereorecta* ash-colored, straight.)

Basonym *Streptomyces cinereorectus* Terekhova and Preobrazhenskaya 1986, 574^{AL}.

The descriptions is that reported in Kämpfer (2012).

The type strain is AS 4.1622^{T}, ATCC 43679^{T}, CGMCC 4.1622^{T}, DSM 41469^{T}, IFO 15395^{T}, INA 5202^{T}, JCM 6916^{T}, NBRC 15395^{T}, NRRL B-16360^{T}. Type strain AS 4.1589^{T}, CGMCC 4.1589^{T}, DOA 1196^{T}, DSM 41424^{T}, IFO 15394^{T}, JCM 3371^{T}, KCTC 9705^{T}, NBRC 15394^{T}, NRRL B-2289^{T}.

Description of *Kitasatospora herbaricolor* comb. nov.

Kitasatospora herbaricolor (her.ba.ri'co.lor. L. n. *herbarius* one skilled in plants, a botanist: L. n. *color* color; N. L. adj. *herbaricolor* grass colored green referring to the grass green diffusible pigment produced by the microorganism on chemically defined media).

Basonym *Streptomyces herbaricolor* Kawato and Shinobu 1959, 114^{AL}.

The descriptions is that reported in Kämpfer (2012).

Type strain AS 4.1849^{T}, AS 4.1887^{T}, ATCC 23922^{T}, CBS 424.61^{T}, CBS 906.68^{T}, CGMCC 4.1849^{T}, CGMCC 4.1887^{T}, DSM 40123^{T}, IFO 12876^{T}, IFO 3838^{T}, IFO 3932^{T}, ISP 5123^{T}, JCM 4138^{T}, JCM 4645^{T}, NBRC 12876^{T}, NBRC 3838^{T}, NBRC 3932^{T}, NCIMB 9837^{T}, NRRL B-3299^{T}, NRRL ISP-5123^{T}, RIA 1126^{T}, RIA 654^{T}, Shinobu 608^{T}, VKM Ac-793^{T}.

Description of *Kitasatospora misakiensis* comb. nov.

Kitasatospora misakiensis (mi.sa.ki.en'sis. N.L. fem. adj. *misakiensis* belonging to misaki (referring to Misakicho, Kanagawa Prefecture, Japan, the source of the soil from which the microorganism was isolated).

Basonym *Streptomyces misakiensis* Nakamura 1961, 86[AL].

Description is that reported in Kämpfer (2012).

Type strain AS 4.1437[T], ATCC 23938[T], CBS 278.65[T], CBS 922.68[T], CGMCC 4.1437[T], DSM 40222[T], IFO 12891[T], ISP 5222[T], JCM 4062[T], JCM 4653[T], KCTC 19951[T], LMG 19369[T], NBRC 12891[T], NCIB 9852[T], NRRL B-2923[T], NRRL ISP-5222[T], RIA 1166[T], VKM Ac-625[T].

Description of *Kitasatospora psammotica* comb. nov.

Kitasatospora psammotica (psam.mo'ti.ca. Gr. n. *psammos* sand; N.L. fem. adj. *psammotica* sandy).

Basonym *Streptomyces psammoticus* Virgilio and Hengeller 1960, 167[AL].

Description is that reported in Kämpfer (2012).

Type strain is AS 4.1465[T], ATCC 14125[T], ATCC 25488[T], CBS 175.61[T], CBS 299.65[T], CBS 916.69[T], CGMCC 4.1465[T], DSM 40341[T], IFO 13076[T], ISP 5341[T], JCM 4434[T], KCTC 19966[T], Lepetit Labs C17190[T], NRRL B-3291[T], NRRL ISP-5341[T], RIA 1268[T], RIA 832[T], VKM Ac-996[T].

Description of *Kitasatospora purpeofusca* comb. nov.

Kitasatospora purpeofusca (pur.pe.o.fus'ca. L. adj. *purpureus* purple; L. adj. *fuscus* dark, tawny; N.L. fem. adj. *purpeofusca* dark purple referring to the color of the vegetative mycelium).

Basonym *Streptomyces purpeofuscus* Yamaguchi and Saburi 1955, 207[AL].

Description is that reported in Kämpfer (2012).

Type strain is ATCC 23952[T], CBS 935.68[T], CGMCC 4.1767[T], CGMCC 4.1999[T], DSM 40283[T], IFO 12905[T], ISP 5283[T], JCM 4156[T], JCM 4665[T], KCTC 19967[T], LMG 20283[T], NBRC 12905[T], NCIMB 9822[T], NRRL B-1817[T], NRRL ISP-5283[T], RIA 1197[T], VKM Ac-1825[T], Yamaguchi H-5080[T].

Description of *Kitasatospora fumigata* comb. nov.

Kitasatospora fumigata (fu.ma.ga'ta. L. fem. part. adj. *fumigata* smoked).

Basonym *Streptomyces chrysomallus* subsp. *fumigatus* Frommer 1959, 202[AL].

Description is that reported in Kämpfer (2012).

Type strain AS 4.1589^T, CGMCC 4.1589^T, DOA 1196^T, DSM 41424^T, IFO 15394^T, JCM 3371^T, KCTC 9705^T, NBRC 15394^T, NRRL B-2289^T.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviation

DDH	DNA–DNA hybridization

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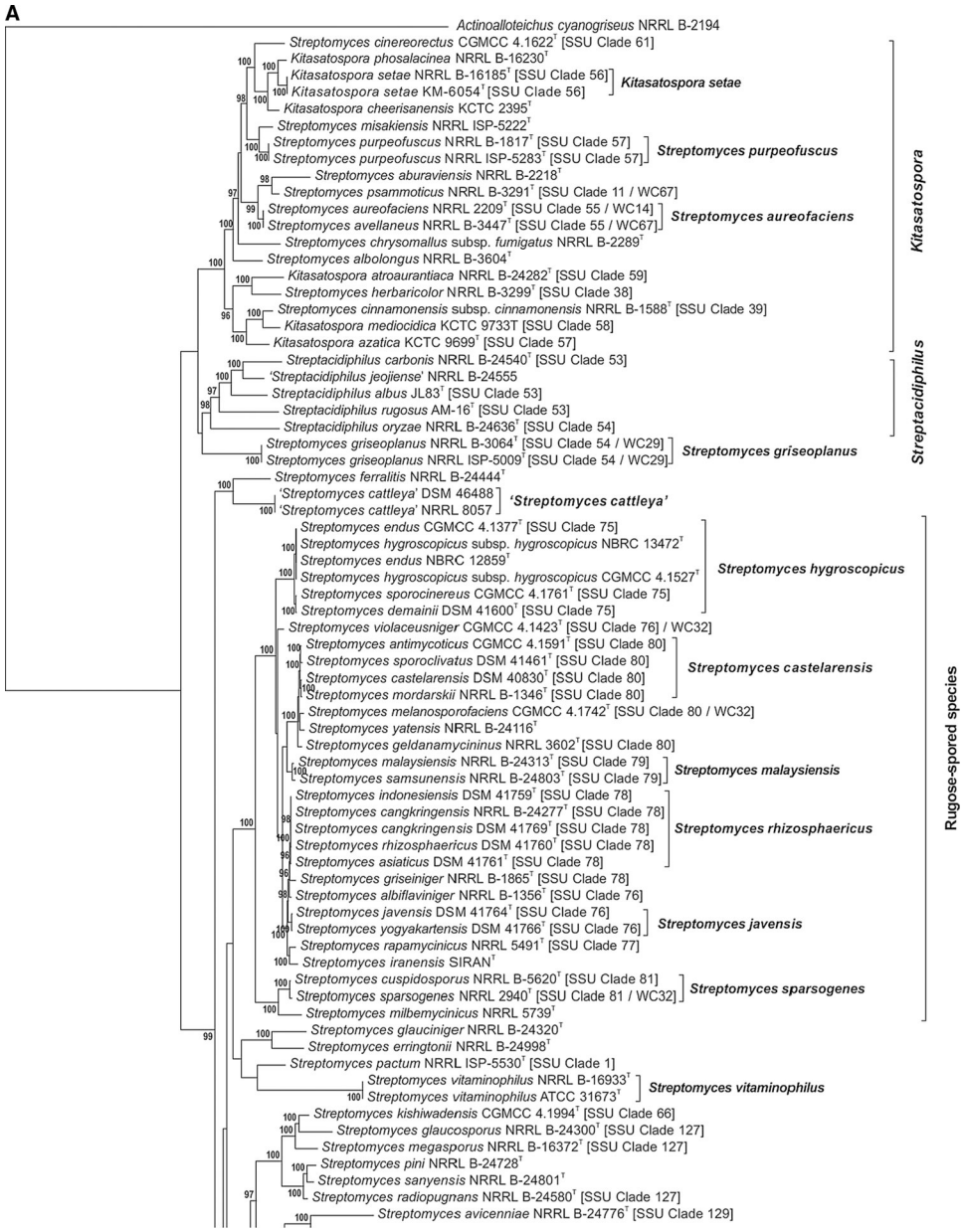

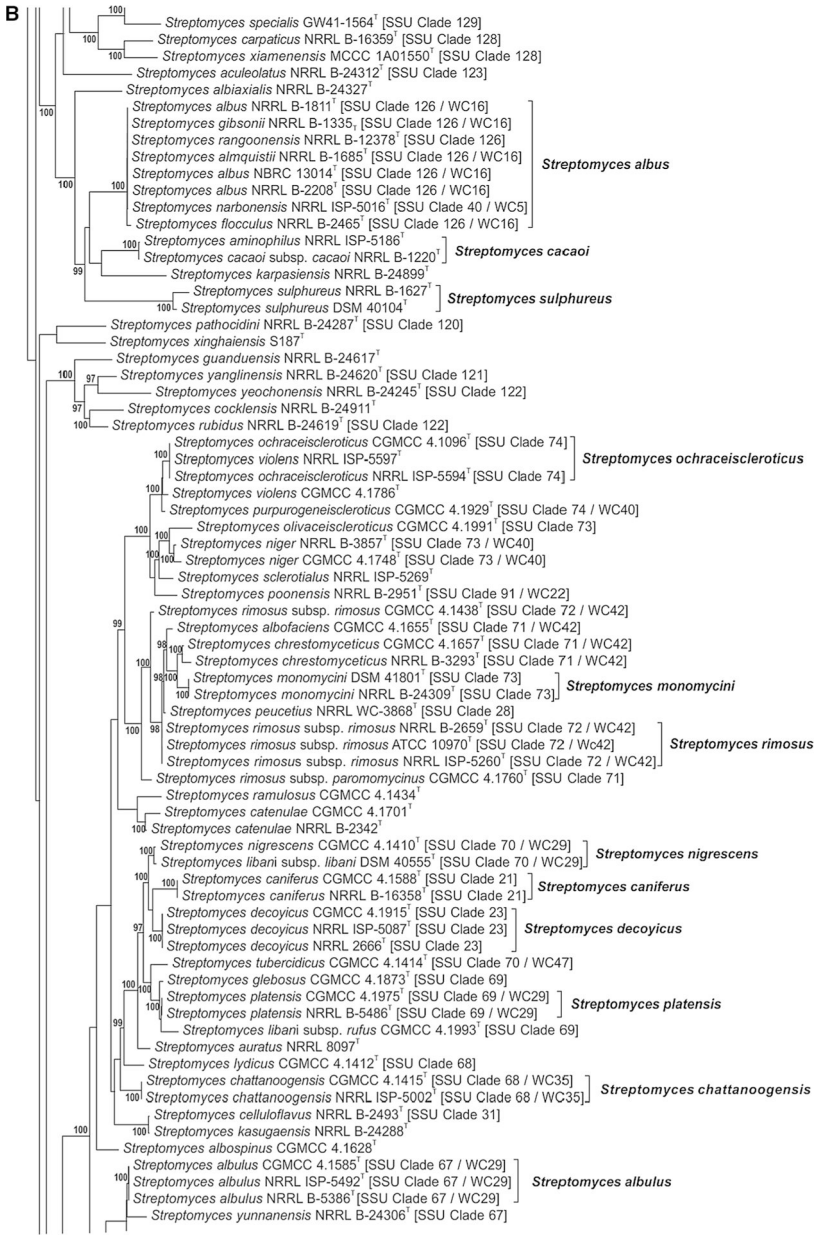

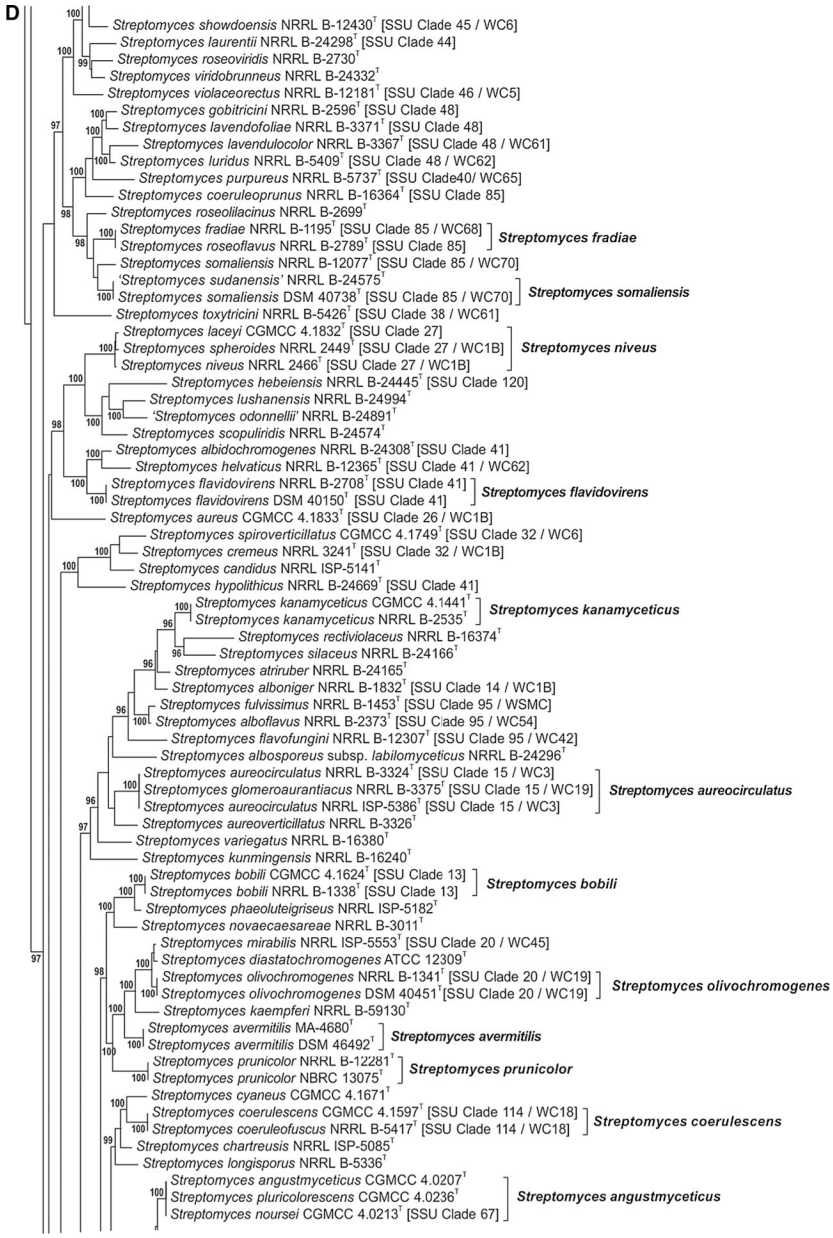

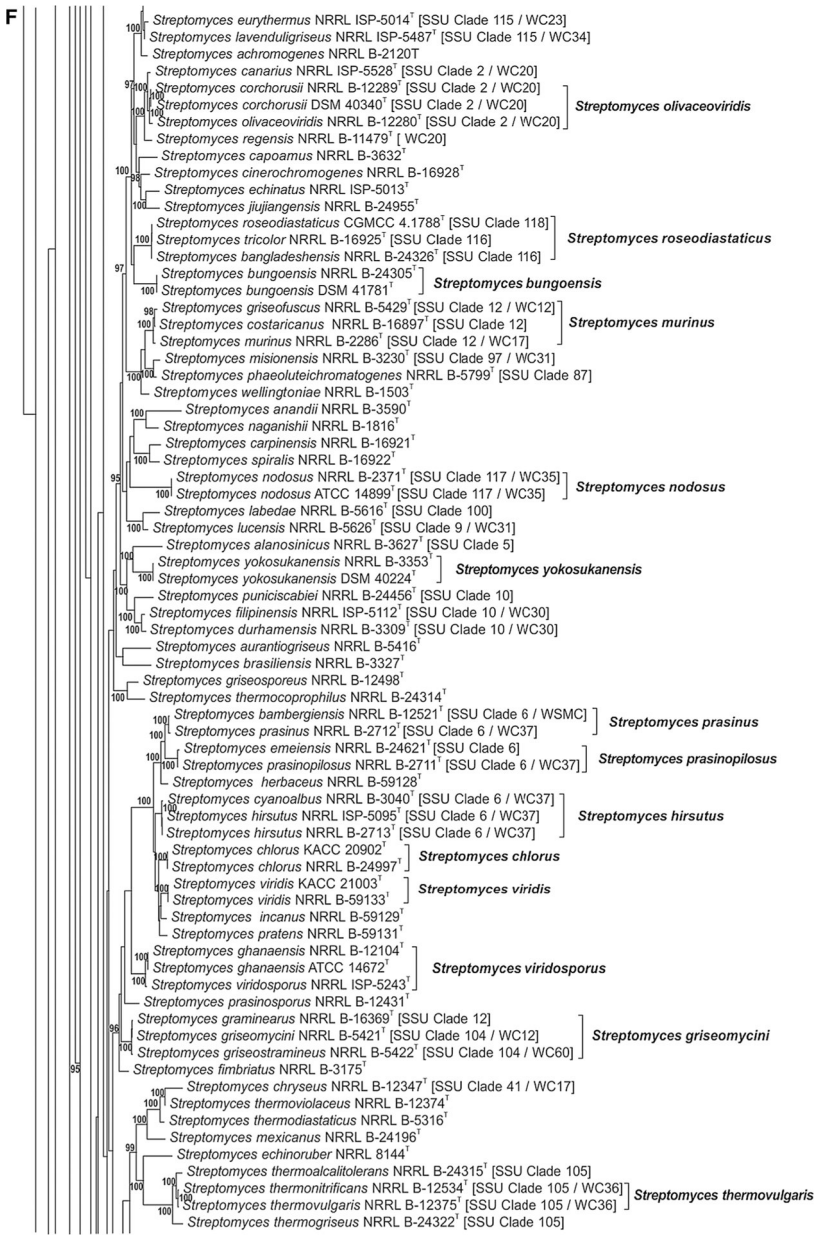

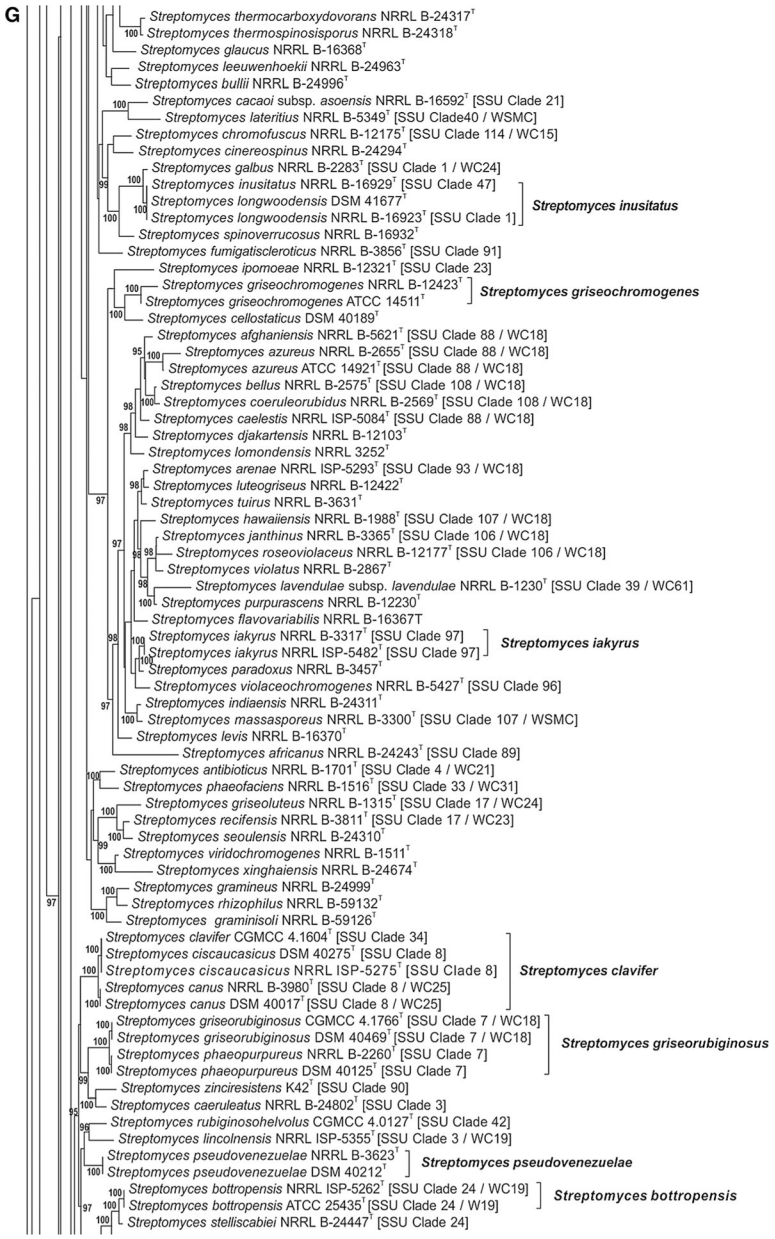

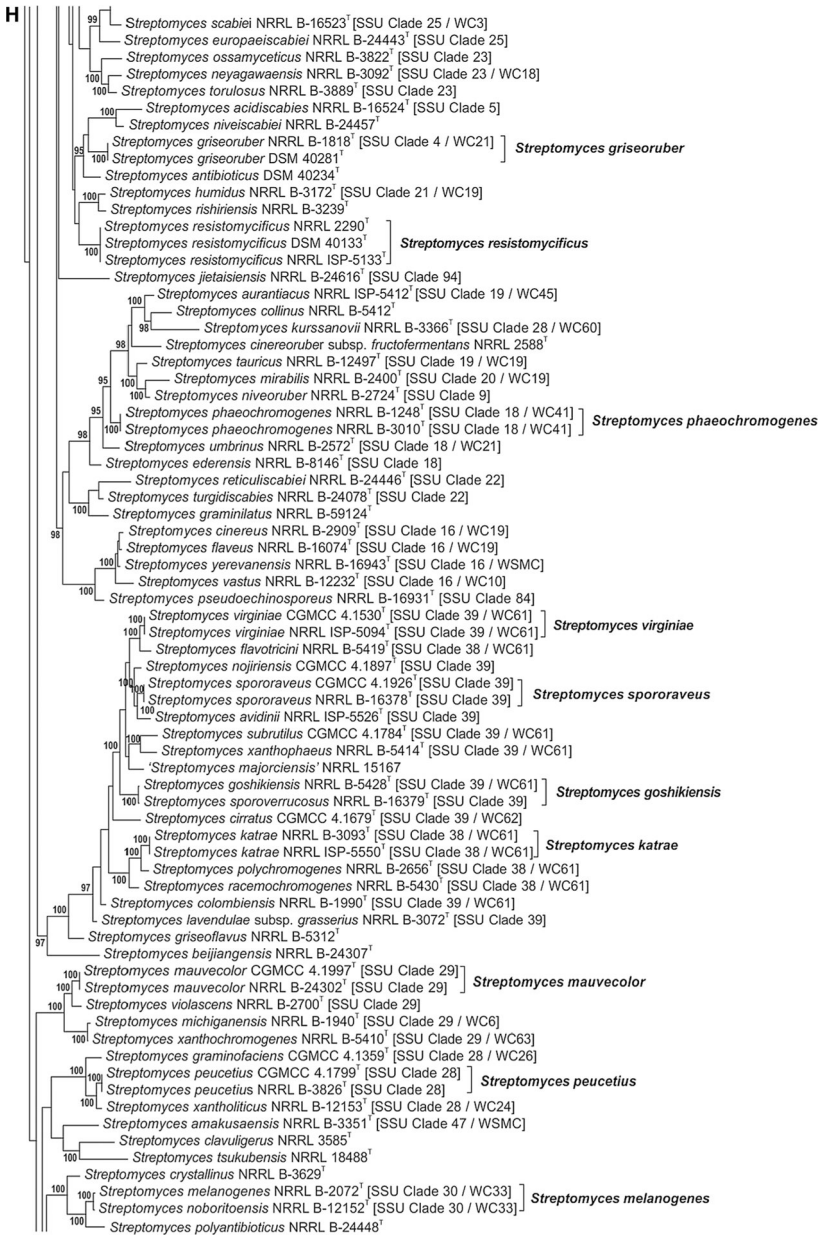

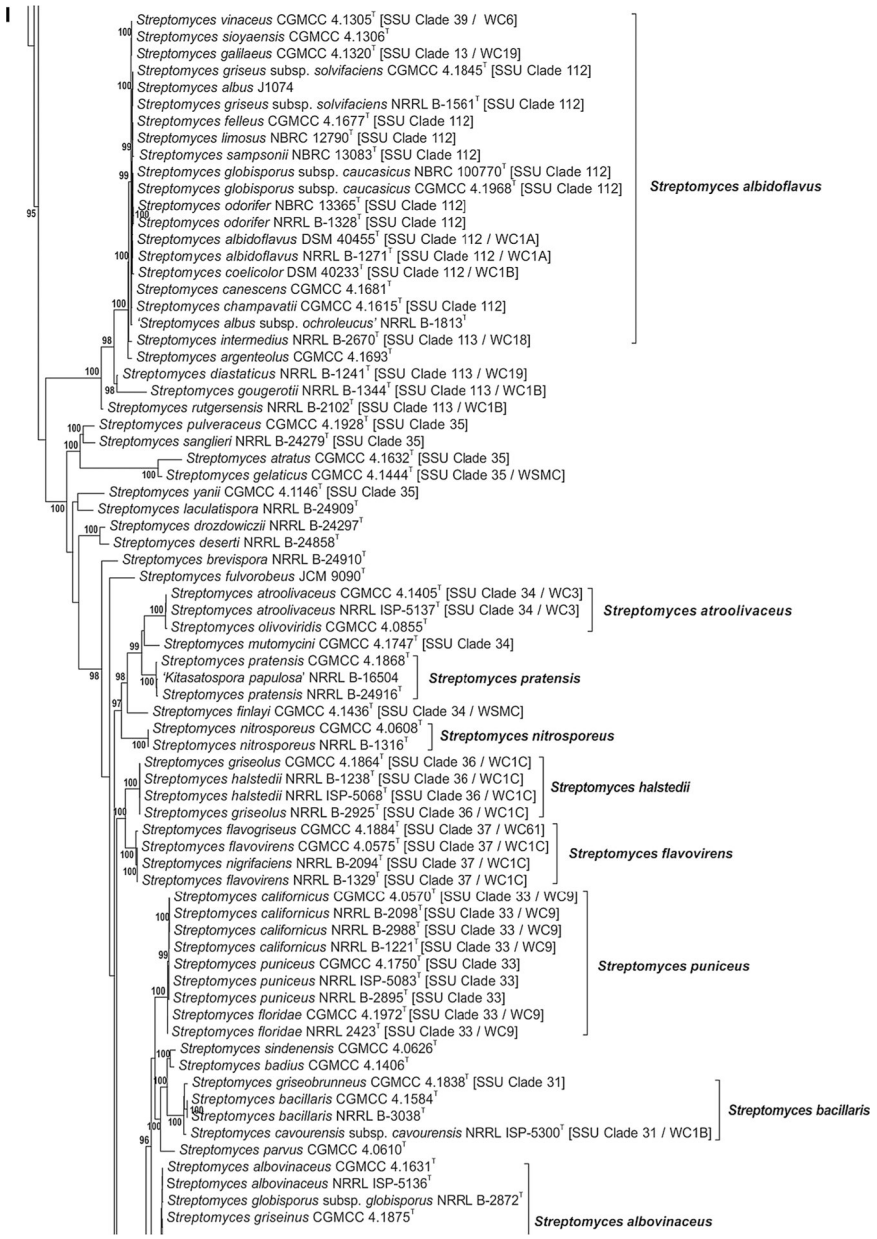

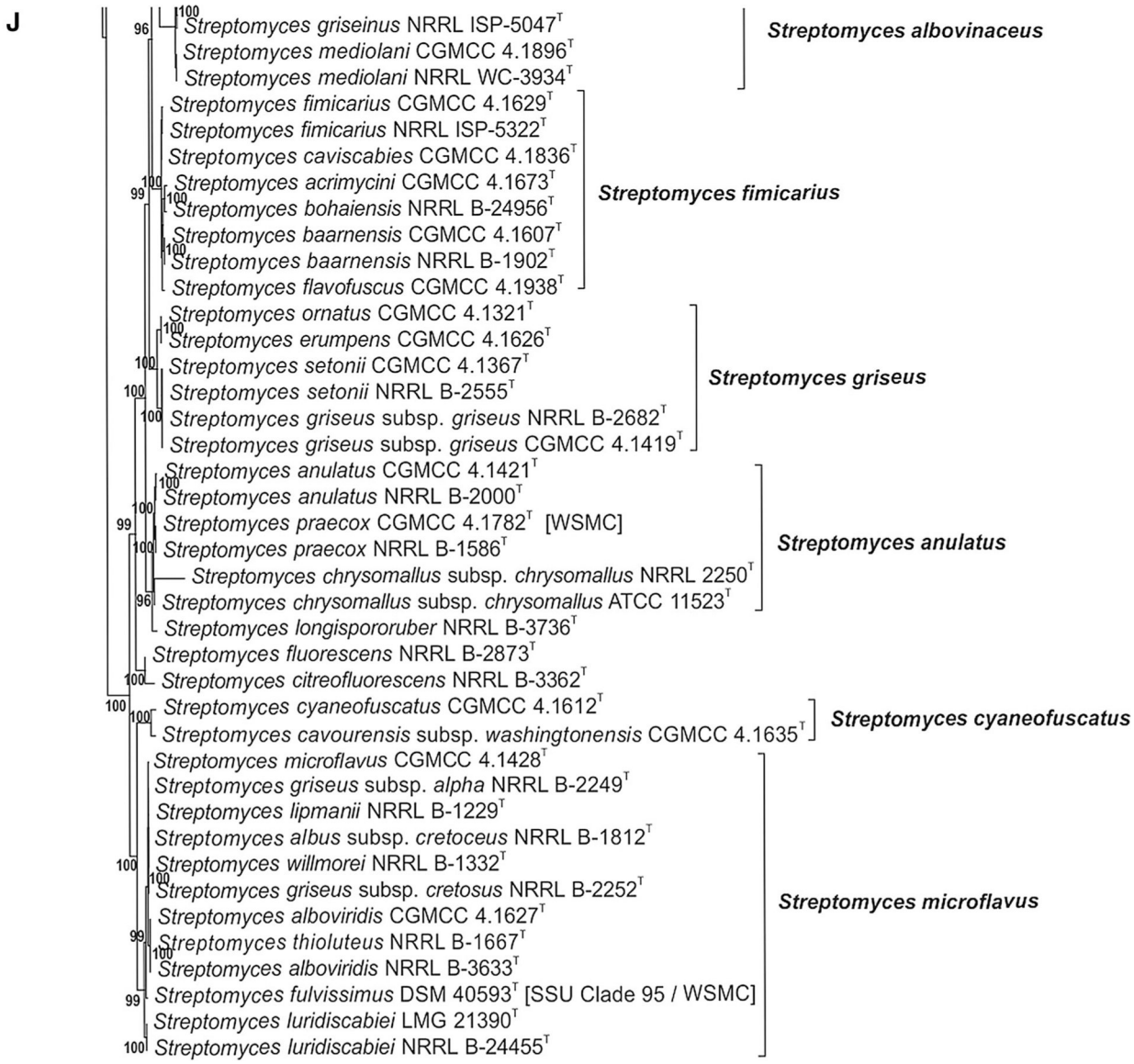

Fig. 1 a–j.

Phylogenetic relationships among strains within the *Streptomycetaceae* were constructed in IQ-Tree version 1.4.2 (Nguyen et al. 2015) using the Maximum Likelihood based on the full partition General Time Reversible model (Nei and Kumar 2000) including invariate sites plus a discrete Gamma model with 4 rate categories (GTR + F + I + G4) with separate branch lengths and models between partitions, which had been determined to be the optimal model considering the 5 gene partitions of these data during the model test phase of analysis. The trees were subjected to 1000 ultrafast bootstrap replications (Minh et al. 2013) and SH-like average likelihood ratio test replications (SH-aLRT) (Guindon et al. 2010). Bootstrap values less than 95% and/or SH-aLRT less than 85% were omitted as suggested by the IQ-Tree developers. Bar scale reflects number of substitutions per sites